

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
9 August 2001 (09.08.2001)

PCT

(10) International Publication Number  
**WO 01/57238 A2**

(51) International Patent Classification<sup>7</sup>: C12Q 1/00

(21) International Application Number: PCT/US01/02510

(22) International Filing Date: 25 January 2001 (25.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
09/497,269 2 February 2000 (02.02.2000) US

(71) Applicant: LIFESCAN, INC. [US/US]; 1000 Gibraltar Drive, Milpitas, CA 95035-6312 (US).

(72) Inventors: YU, Yeung, Siu; 3158 Paseo Robles, Pleasanton, CA 94560 (US). SHAH, Mahesh; 1759 Jones, Santa Clara, CA 95051 (US).

(74) Agent: FIELD, Bret, E.; Bozicevic, Field & Francis LLP, Suite 200, 200 Middlefield Road, Menlo Park, CA 94025 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

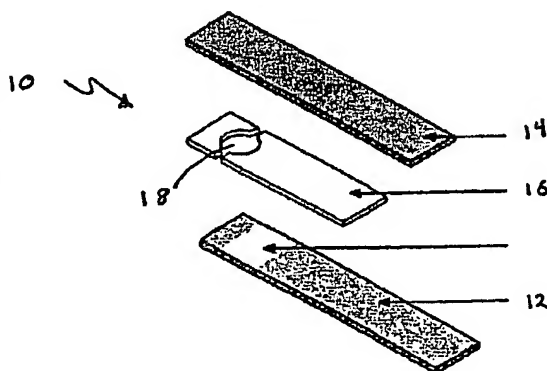
**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ELECTROCHEMICAL TEST STRIP FOR USE IN ANALYTE DETERMINATION

WO 01/57238 A2



(57) Abstract: Electrochemical test strips and methods for their use in the detection of an analyte in a physiological sample are provided. The subject test strips have a reaction zone defined by opposing metal electrodes separated by a thin spacer layer. The metal surface of at least one of the electrodes is modified by a homogenous surface modification layer made up of linear self-assembling molecules having a first sulfhydryl end group and a second sulfonate end group separated by a short chain alkyl linking group, where 2-mercaptoethane sulfonic acid or a salt thereof is preferred in certain embodiments. The subject electrochemical test strips find application in the detection of a wide variety of analytes, and are particularly suited for use the detection of glucose.

BEST AVAILABLE COPY

hydrophilic surface that provided rapid wicking time and did not interfere with the electrochemical measurements of the electrode.

#### Relevant Literature

U.S. Patent documents of interest include: 5,834,224; 5,942,102 and 5,972,199.

- 5 Other patent documents of interest include WO 99/49307; WO 97/18465 and GB 2 304 628. Other references of interest include: Dalmia et al, J. Electroanalytical Chemistry (1997) 430: 205-214; Nakashima et al., J. Chem. Soc. (1990) 12: 845-847; and Palacin et al., Chem. Mater. (1996) 8:1316-1325.

### 10 SUMMARY OF THE INVENTION

- Electrochemical test strips and methods for their use in the detection of an analyte, e.g. glucose, in a physiological sample, e.g. blood, are provided. The subject test strips have a reaction area defined by opposing metal electrodes separated by a thin spacer layer. The metal surface of at least one of the electrodes is modified by a homogenous surface
- 15 modification layer made up of linear self-assembling molecules having a first sulfhydryl end group and a second sulfonate end group separated by a short chain alkyl linking group, where 2-mercaptoethane sulfonic acid or a salt thereof is preferred in certain embodiments. The subject electrochemical test strips find application in the detection of a wide variety of analytes, and are particularly suited for use the detection of glucose.

20

### BRIEF DESCRIPTION OF THE FIGURES

Figs. 1 and 2 provide a representation of an electrochemical test strip according to the subject invention.

- Fig. 3 provides an analysis of the contact angle of various cystine treated metallic
- 25 electrodes at various times following treatment.

Fig. 4 provides an analysis of the wicking time of various cystine treated metallic electrodes at various times following treatment.

Figs. 5A and 5B provide an analysis of the contact angle of various MESA treated metallic electrodes at various times following treatment.

- Fig. 6 provides an analysis of the wicking time of various MESA treated metallic
- 30 electrodes at various times following treatment.

Fig. 7 provides a comparison of the wicking time of various cystine and MESA coated electrodes.

elements, i.e. the working and reference electrodes, the spacer layer and the reaction area are now described separately in greater detail.

### *Electrodes*

5           As indicated above, the subject electrochemical test strips include a working electrode and a reference electrode. Generally, the working and reference electrodes are configured in the form of elongated rectangular strips. Typically, the length of the electrodes ranges from about 1.9 to 4.5cm, usually from about 2 to 2.8cm. The width of the electrodes ranges from about 0.38 to 0.76cm, usually from about 0.51 to 0.67cm. The reference  
10       electrodes typically have a thickness ranging from about 10 to 100nm and usually from about 18 to 22nm. In certain embodiments, the length of one of the electrodes is shorter than the length of the other electrode, wherein in certain embodiments it is about 0.32cm shorter.

          The working and reference electrodes are further characterized in that at least the surface of the electrodes that faces the reaction area in the strip is a metal, where metals of  
15       interest include palladium, gold, platinum, silver, iridium, carbon, doped indium tin oxide, stainless steel and the like. In many embodiments, the metal is gold or palladium. While in principle the entire electrode may be made of the metal, each of the electrodes is generally made up of an inert support material on the surface of which is present a thin layer of the metal component of the electrode. In these more common embodiments, the thickness of the  
20       inert backing material typically ranges from about 51 to 356  $\mu\text{m}$ , usually from about 10 to 153  $\mu\text{m}$  while the thickness of the metal layer typically ranges from about 10 to 100nm and usually from about 20 to 40nm, e.g. a sputtered metal layer. Any convenient inert backing material may be employed in the subject electrodes, where typically the material is a rigid material that is capable of providing structural support to the electrode and, in turn, the  
25       electrochemical test strip as a whole. Suitable materials that may be employed as the backing substrate include plastics, e.g. PET, PETG, polyimide, polycarbonate, polystyrene, silicon, ceramic, glass, and the like.

          The subject test strips are further characterized in that at least one of the metallic surfaces of the electrodes, and in some embodiments both of the metallic surfaces of the  
30       electrodes, that face, i.e. border or bound, the reaction area, have a surface modification layer present thereon. The surface modification layer is a homogenous layer of self-assembling molecules that renders the surface stably hydrophilic in a storage stable manner. More specifically, the surface modification layer should impart to the surface a low contact angle,

and more usually from about 0.05 to 0.3%. Contact is maintained for a sufficient period of time for the monolayer to form, e.g. for a period of time ranging from about 0.5 to 3 minutes, usually from about 0.5 to 2 min, followed by drying of the electrode surface for use in the subject electrochemical test strips. A more detailed representative fabrication profile is  
5 provided in the experimental section, *infra*.

### *Spacer Layer*

A feature of the subject electrochemical test strips is that the working and reference electrodes as described above face each other and are separated by only a short distance,  
10 such that the distance between the working and reference electrode in the reaction zone or area of the electrochemical test strip is extremely small. This minimal spacing of the working and reference electrodes in the subject test strips is a result of the presence of a thin spacer layer positioned or sandwiched between the working and reference electrodes. The thickness of this spacer layer generally ranges from about 1 to 500um, usually from about  
15 102 to 153um. The spacer layer is cut so as to provide a reaction zone or area with at least an inlet port into the reaction zone, and generally an outlet port out of the reaction zone as well. A representative spacer layer configuration can be seen in Figs. 1 and 2. While the spacer layer is shown in these figures as having a circular reaction area cut with side inlet and outlet vents or ports, other configurations are possible, e.g. square, triangular, rectangular, irregular  
20 shaped reaction areas, etc. The spacer layer may be fabricated from any convenient material, where representative suitable materials include PET, PETG, polyimide, polycarbonate and the like, where the surfaces of the spacer layer may be treated so as to be adhesive with respect to their respective electrodes and thereby maintain the structure of the electrochemical test strip. Of particular interest is the use of a die-cut double-sided adhesive  
25 strip as the spacer layer.

### *Reaction Zone*

The subject electrochemical test strips include a reaction zone or area that is defined by the working electrode, the reference electrode and the spacer layer, where these elements  
30 are described above. Specifically, the working and reference electrodes define the top and bottom of the reaction area, while the spacer layer defines the walls of the reaction area. The volume of the reaction area is at least about 0.1µl, usually at least about 1 µl and more usually at least about 1.5µl, where the volume may be as large as 10µl or larger. As

The redox reagent system is generally present in dry form. The amounts of the various components may vary, where the amount of enzyme component typically ranges from about 0.1 to 10% by weight.

## 5 METHODS

Also provided by the subject invention are methods of using the subject electrochemical test strips to determine the concentration of an analyte in a physiological sample. A variety of different analytes may be detected using the subject test strips, where representative analytes include glucose, cholesterol, lactate, alcohol, and the like. In many  
10 preferred embodiments, the subject methods are employed to determine the glucose concentration in a physiological sample. While in principle the subject methods may be used to determine the concentration of an analyte in a variety of different physiological samples, such as urine, tears, saliva, and the like, they are particularly suited for use in determining the concentration of an analyte in blood or blood fractions, and more particularly in whole  
15 blood.

In practicing the subject methods, the first step is to introduce a quantity of the physiological sample into the reaction area of the test strip, where the electrochemical test strip is described *supra*. The amount of physiological sample, e.g. blood, that is introduced into the reaction area of the test strip may vary, but generally ranges from about 0.1 to 10ul,  
20 usually from about 1 to 1.6ul. The sample may be introduced into the reaction area using any convenient protocol, where the sample may be injected into the reaction area, allowed to wick into the reaction area, and the like, as may be convenient.

Following application of the sample to the reaction zone, an electrochemical measurement is made using the reference and working electrodes. The electrochemical  
25 measurement that is made may vary depending on the particular nature of the assay and the device with which the electrochemical test strip is employed, e.g. depending on whether the assay is coulometric, amperometric or potentiometric. Generally, the electrochemical measure will measure charge (coulometric), current (amperometric) or potential (potentiometric), usually over a give period of time following sample introduction into the  
30 reaction area. Methods for making the above described electrochemical measurement are further described in U.S. Patent Nos.: 4,224,125; 4,545,382; and 5,266,179; as well as WO 97//18465; WO 99/49307; the disclosures of which are herein incorporated by reference.

## EXPERIMENTAL

### I. Preparation of Electrochemical Test Strips

#### A. Preparation of MESA Treated Electrochemical Test Strips

A (0.1)1% 2-mercaptoethane sulfonic acid (MESA) solution is prepared by  
5 dissolving 1.000 gm MESA (TCI, Catalog # M0913) into 999 gm Milli Q water. Gold and  
palladium sheets are prepared by sputtering the surface of a 7 mil thick polyester substrate  
with gold or palladium such that a surface metallic layer of 100 to 500 angstroms is  
obtained. Following preparation of these gold and palladium master rolls, 12 in x 8.5 inch  
sheets are cut. The sheets are then immersed in the 1% MESA solution for 1 minute. The  
10 coated sheet is then air dried for 1 hour and tested for contact angle using a Goniometer and  
water as described in Procedure A found in Appendix A, *infra*, to ensure that the contact  
angle is  $< 20^\circ$ .

Test strips having dimensions of 0.2 x 1.2 inch are then cut from the above gold and  
metal sheets and are used to fabricate electrochemical test strips as follows. A gold strip and  
15 palladium strip are used to sandwich a die-cut double sided pressure sensitive adhesive strip  
having a thickness of 0.005 in and a circular die-cut area that defines the reaction zone, inlet  
and outlet ports when sandwiched between the gold and metal strips, as shown in Figs. 1 and  
2. A dry reagent consisting of buffer, mediator, enzyme and bulking agents is ink jetted onto  
the palladium electrode prior to sandwiching the double-sided adhesive.

20

#### B. Preparation of Cystine Treated Electrochemical Test Strips

Cystine treated electrochemical test strips were prepared according to a standard  
industry protocol.

### 25 II. Characterization of Cystine Treated Electrochemical Test Strips

#### A. Contact Angle

The contact angle of cystine treated gold and palladium test strips was determined  
with water and a goniometer as described in Procedure B found in Appendix A, *infra*. The  
contact angle was determined at various times following surface treatment, i.e. 0, 7 and 14  
30 days following treatment, and at various storage temperatures, e.g. room temperature and 56  
°C. The results are provided in Fig. 3.

The above results and discussion demonstrate that significantly improved electrochemical test strips for use in the determination of an analyte in a test sample are provided by the subject invention. Specifically, storage stable electrochemical test strips having durable hydrophilic surfaces that exhibit low interference to the electrochemical measurement of oxidized species and have fast wicking times are provided. Furthermore, the surface modifying reagents used to modify the surfaces of the subject test strips are odorless. As such, the subject invention represents a significant contribution to the art.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

6. Adobe Premiere software 4.2 for video capture
7. Computer System and a Monitor
8. Two side adhesive tape & a platform for strip

5    **Procedure:**

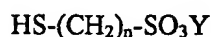
1. Place a strip on a platform and hold it with tape.
2. Place the strip under the camera lens and adjust the focus and magnification.
3. Launch the Premiere software and open movie captures program. Select 30fps NTSC system for capturing live movie.
- 10    4. Place 5 uL of 70 % hct blood on Parafilm surface.
5. Turn on recording mode and apply blood from either side of test strip in to the capillary.
6. Turn off the recording mode when blood reaches the other end of test strip
7. Go to the image window and analyze it. Use In mark when blood touches the strip and out mark when blood reaches the other end. Software does the frames count (30 frames/seconds) and displays in lower window.
- 15    8. To calculate wicking time, divide number of frames with 30.
9. Repeat the procedure for # of strips

20



6. An electrochemical test strip comprising:
- (a) a reaction zone defined by opposing working and reference electrodes comprising a metal surface separated by a thin spacer layer, wherein at least one of said first and second metallic electrodes has a surface modified with a homogenous surface modification layer made up of self assembling molecules of the formula:
- $$\text{HS}-(\text{CH}_2)_n-\text{SO}_3\text{Y}$$
- wherein:
- n is an integer from 1 to 6; and
- Y is H or a cation; and
- (b) a redox reagent system present in said reaction zone, wherein said redox reagent system comprises enzymes and a mediator.
7. The electrochemical test strip according to Claim 6, wherein said reaction zone has a volume ranging from about 0.1 to 10  $\mu\text{l}$ .
8. The electrochemical test strip according to Claim 6, wherein said metal is selected from the group consisting of gold and palladium.
9. The electrochemical test strip according to Claim 6, wherein said enzymes include an oxidizing enzyme.
10. The electrochemical test strip according to Claim 9, wherein said oxidizing enzyme is a glucose oxidizing enzyme.
11. The electrochemical test strip according to Claim 6, wherein said self assembling molecule is 2-mercaptoethane sulfonic acid or a salt thereof.
12. An electrochemical test strip for use in detecting the concentration of glucose in a physiological sample, said test strip comprising:
- (a) a reaction zone defined by opposing working and reference electrodes comprising a metal surface selected from the group consisting of gold and palladium separated by a thin spacer layer, wherein at least one of said first and second metallic

17. The method according to Claim 16, wherein said self-assembling molecules have the formula:



wherein:

- 5                   n is an integer from 1 to 6; and  
                  Y is H or a cation.

18. The method according to Claim 17, wherein said self-assembling molecules are 2-mercaptoethane sulfonic acid or a salt thereof.

10

19. The method according to Claim 16, wherein said analyte is glucose.

20. The method according to Claim 19, wherein said redox reagent system comprises a glucose oxidizing enzyme.

15

21. A kit for use in determining the concentration of an analyte in a physiological sample, said kit comprising:

(a) an electrochemical test strip comprising:

20                   (i) a reaction zone defined by opposing working and reference metallic electrodes separated by a spacer layer, wherein at least one of said first and second metallic electrodes has a surface modified with a homogenous surface modification layer made up of self assembling molecules having a first sulfhydryl end group and a second sulfonate end group, wherein said sulfhydryl and sulfonate end groups are separated by a lower alkyl linker group; and

25

(ii) a redox reagent system present in said reaction zone, wherein said redox reagent system comprises at least one enzyme and a mediator; and

(b) at least one of:

- 30                   (i) a means for obtaining said physiological sample; and  
                  (ii) an analyte standard.

22. The kit according to Claim 21, wherein said analyte is glucose.

1/4

FIG. 1

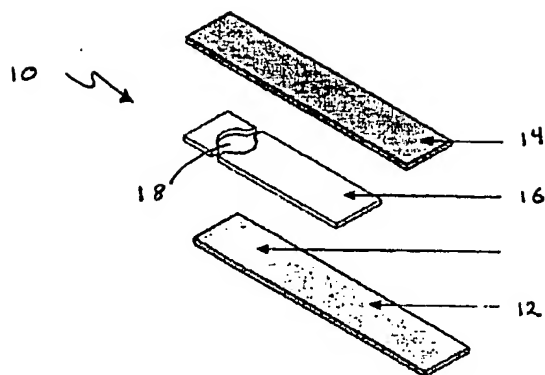
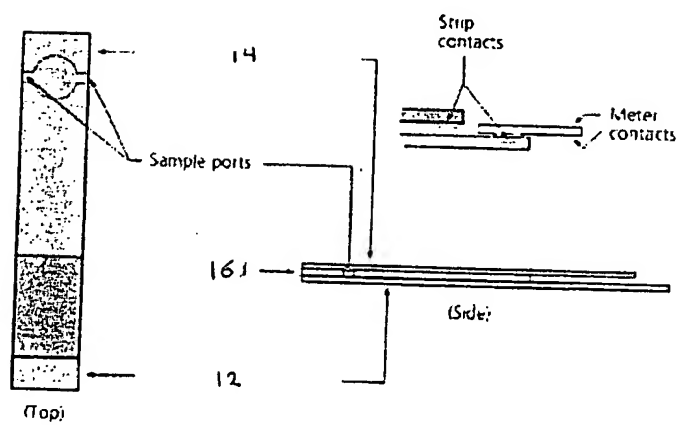


FIG. 2



3/4

FIG. 5A

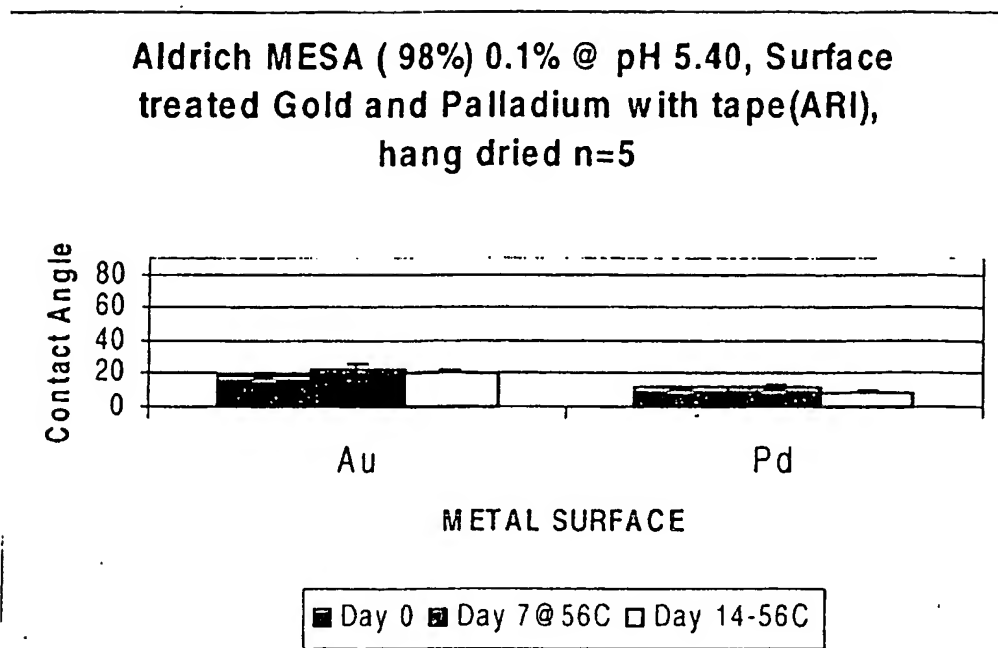
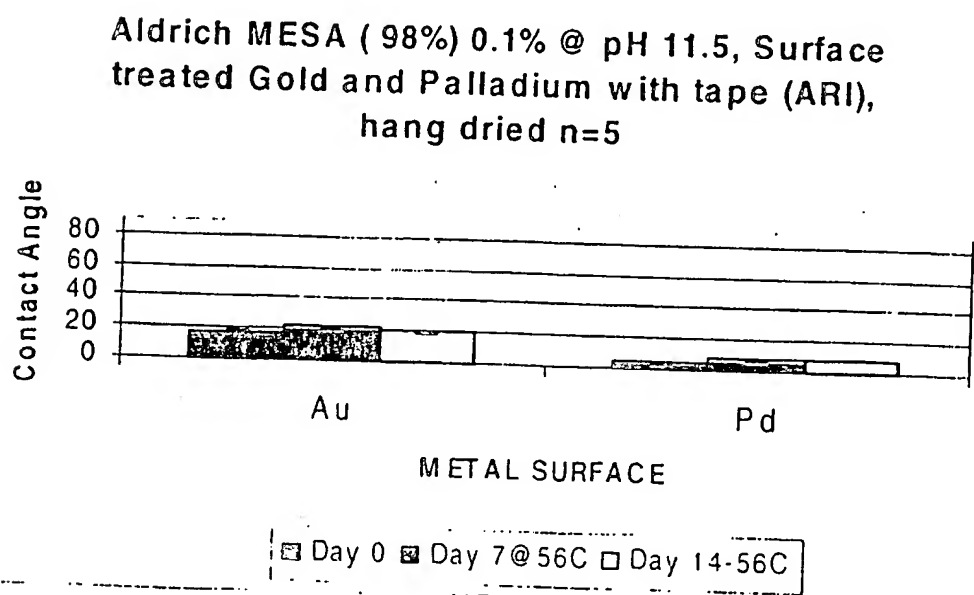


FIG. 5B



**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☒ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**